**Journal of Sciences, Islamic Republic of Iran 19(3): 217-221 (2008)** http://jsciences.ut.ac.ir
University of Tehran, ISSN 1016-1104

# Synthesis and Pharmacological Evaluation of Some Novel Isatin Derivatives for Antimicrobial Activity

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## Abstract

In the present work, a series of new 5-substituted-3-(4-arylimino)-1-[5-mercapto(1,3,4-oxadiazolyl)]-methyl-indol-2-one **(4a-g)** have been synthesized by heterocyclization of 5-substituted-3-(4-arylimino)-2-oxo-1-indole acetylhyrazide **(3a-g)** on treatment with $CS_2$ in ethanolic KOH. The compound **4a** was characterized by its elemental analysis, IR, $^1$HNMR and Mass Spectroscopy. The synthesized compounds **(4a-g)** were evaluated for *in vitro* antibacterial activity and antifungal activity against various strains of bacteria and fungi.

**Keywords:** Isatin; 1,3,4-Oxadiazole; Antibacterial; Antifungal

## Introduction

The synthesis of a newer class of anti-bacterial and anti-fungal agents is in need of time, especially against drug-resistant bacteria and fungi, such as gram-positive and gram-negative strains, which are responsible for a number of serious infections in the acute and chronic care units in hospitals. Literature survey revealed that isatin possess diverse chemotherapeutic activities such as antibacterial [1], antifungal [2], antiviral [3], anti-HIV [4], anti-mycobacterial [5], anti cancer [6], anti-inflammatory [7] and anticonvulsant [8]. 1,3,4-oxadiazole were seen to possess many activities such as antibacterial [9], antifungal [10], antiviral [11], anti-mycobacterial [12], anti-cancer [13], anti-inflammatory [14] and anticonvulsant [15]. The study of above pharmacophores reveals that the combination of these two entities may result in increased antimicrobial activity.

In view of biological importance of these two moieties, it was planned to synthesize a new series of isatin containing oxadiazole derivatives and to evaluate the new compounds for their anti-microbial activity.

## Material and Methods

### *Experimental*

All solvents, reagents and catalysts were of analytical grade and used without further purification. The melting points were determined by open capillary method and were uncorrected. The purity of compounds was confirmed by thin layer chromatography using Silica Gel glass plates as the stationary phase and with suitable mobile phase. IR spectra were recorded using KBr on FTIR-8400S Shimadzu. $^1$H NMR spectra were recorded on Joel-FT-NMR-300MHz using DMSO-$d_6$ as solvents and TMS as internal standard. Mass spectra were recorded on Shimadzu QP-2010 GC-MS. The elemental analysis was performed on Perkin-Elmer series 2400. The synthesis of isatin derivatives were prepared as

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reported in the literature [16].

Synthetic route of 5-substitued-3-(4-arylimino)-1-[5-mercapto(1,3,4-oxadiazolyl)]-methyl-indol-2-one analogs is given in Figure 1.

### *Synthesis of 3-(4-Fluorophenylimino)-1H-indol-2-one (1) [17]*

A mixture of indole-2, 3-dione (1.47g, 0.01M) and 4-fluoroaniline (1.11ml, 0.01M) in absolute ethanol (20ml) was refluxed for 0.5 h in the presence of 2-3 drops of glacial acetic acid. After cooling, was filtered and recrystallised from ethanol to give 1.05 g of compound 1.

Yield: 71.6 %; m. p. 210-216 °C; IR (KBr): 3446, 1614, 1743 $cm^{-1}$.

### *Synthesis of 3-(4-fluorophenylimino)-2-oxo-1-indole-ethylacetate (2) [18]*

A mixture of 3-(4-fluorophenylimino)-1*H*-indol-2,3-dione **(1)** (2.40 g, 0.01M), ethyl chloroacetate (1.22 ml, 0.01M) and potassium carbonate(2.2g, 0.015M) in dry acetone was refluxed for 20 h. The reaction mixture was poured onto crushed ice, and the solid was then filtered, washed with water and recrystallised from methanol to give 1.53 g of compound 2. Yield: 64%; m. p. 105-106 °C; IR (KBr): 1330 , 1614, 1724, 1606 $cm^{-1}$.

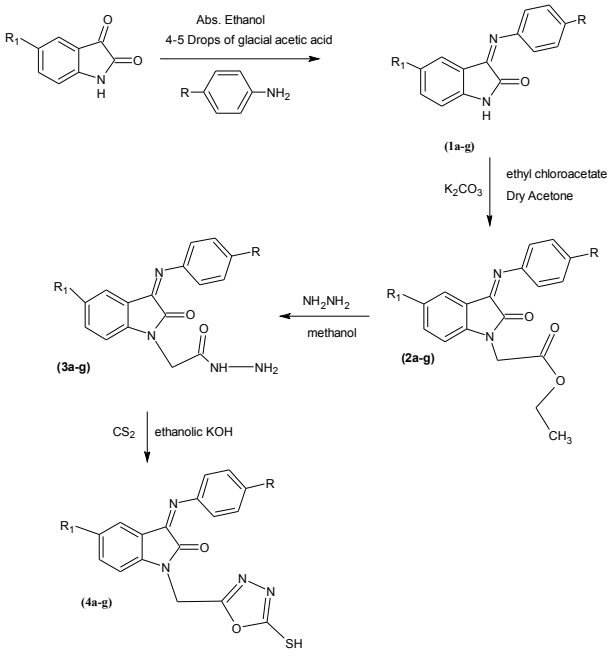



**Figure 1.** Synthesis of 5-substituted-3-(4-arylimino)-1-[5-mercapto (1,3,4 - oxadiazolyl)]-methyl-indol-2-one analogs.

### *Synthesis of 3-(4-fluorophenylimino)-2-oxo-1-indole-acetylhydrazide (3) [19]*

A mixture of 3-(4-fluorophenylimino)-2-oxo-1-indole-ethylacetate **(2)** (3.26g, 0.01M) and hydrazine hydrate (99%, 0.5ml, 0.01M) in methanol (20ml) was refluxed for about 5 h on steam bath. After completion of reaction (monitored by TLC), the mixture was cooled and the resulting solid was filtered, dried and recrystallised from ethanol to give 2.02 g of compound 3. Yield: 62%; m. p. 225-226 °C; IR (KBr): 1330, 3398, 1614, 1743, 1606 $cm^{-1}$.

### *Synthesis of 3-(4-fluorophenylimino)-1-[5-mercapto (1,3,4-oxadiazolyl)]-methyl-indol-2-one (4) [20]*

To a cooled solution of 3-(4-fluorophenylimino)-2-oxo-1-indole-acetylhydrazide (3.12g, 0.01M) in ethanolic KOH (15 ml) to 0 °C added $CS_2$ (0.75ml, 0.01M). The mixture was refluxed for 8 h and allowed to stand at room temperature overnight. The mixture was then concentrated to a small volume, water was added to it and neutralized with 1*N* HCl and the organics were extracted with ethyl acetate. The organic layer was then dried over sodium sulfate, filtered and concentrated under reduced pressure to afford 2.62 g of compound 4. Yield: 84%; m. p. 125 °C; IR (KBr): 1060, 1300, 1614, 1662, 2582 $cm^{-1}$.

### *Spectral Data of Representative Compound 4a*

IR (KBr): 1060, 1342, 1614, 1662, 2582 $cm^{-1}$; $^1$HNMR (DMSO - $d_6$, δ : 3.49 (s, H, -SH), 5.14 (s, 2H, N-$CH_2$), 6.72-7.43 (m, 8H, Ar-H); MS (m/z): 354, 246, 131 , 115 , 108 , 101 , 90 , 76 (100%).

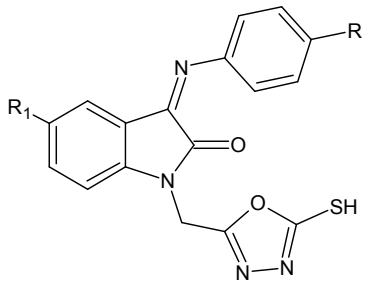
Other isatin derivatives were prepared similarly. The physical constants are presented in Table 1.

### *Biological Evaluation*

*In vitro Antibacterial & Antifungal Activity*

All the compounds were evaluated for their *in-vitro* anti-bacterial activity against *S. aureus NCIM 2079*, *B. subtilis ATCC 6633*, *E. coli ATCC M 200, P. vulgaris NCIM 2813* and anti-fungal against *C. albicans NCIM 3471, A. niger NCIM 545* standard strains using disc diffusion method [21] which were procured from the Department of Microbiology, R.C. Patel College of Arts, Science and Commerce, Shirpur, Dist Dhule, India. Each disc contains 200 μg/ml and 500 μg/ml of the tested compounds. Paper Disc Diffusion method was performed using Mueller-Hinton (Hi-Media) Agar (anti-bacterial) and Potato Dextrose (Hi-Media) Agar (anti-

**Table 1.** Physical data of 5-substituted-3-(4-arylimino)-1-[5-mercapto (1, 3, 4 - oxadiazolyl)]-methyl-indol-2-one analogs



| Sample code | R | $R_1$ | Molecular formula | MW (gm) | Melting point (°C) | Elemental analysis (%), Calculated (Found) | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | C | H | N |
| 4a | F | H | $C_{17}H_{11}N_4O_2SF$ | 354 | 125-128 | 57.6 (57.62) | 3.05 (3.10) | 14.76 (15.81) |
| 4b | F | Cl | $C_{17}H_{11}N_4O_2SFCl$ | 388 | 125-130 | 52.51 (52.57) | 2.53 (2.57) | 14.47 (14.43) |
| 4c | F | F | $C_{17}H_{11}N_4O_2SF_2$ | 372 | 130-136 | 54.85 (54.83) | 2.65 (2.68) | 15.07 (15.05) |
| 4d | F | $CH_3$ | $C_{18}H_{13}N_4O_2SF$ | 368 | 180-185 | 58.71 (58.69) | 3.51 (3.53) | 15.14 (15.21) |
| 4e | F | Br | $C_{17}H_{11}N_4O_2SFBr$ | 433 | 138-145 | 47.04 (47.11) | 2.25 (2.30) | 12.85 (12.93) |
| 4f | Cl | H | $C_{17}H_{11}N_4O_2SCl$ | 370 | 134-138 | 54.86 (54.98) | 3.01 (2.96) | 15.07 (15.09) |
| 4g | Br | H | $C_{17}H_{11}N_4O_2SBr$ | 415 | 180-192 | 49.06 (49.15) | 2.59 (2.65) | 13.44 (13.49) |

fungal). Suspensions of each microorganism were prepared to contain approximately $10^6$ colony forming units (cfu)/ml and applied to plates. The surface of the medium was allowed to dry. The 200 µg/ml and 500 µg/ml (in DMSO) compound impregnated discs were applied to the surface of inoculated plates. The Petri plates were incubated at 37 °C for antibacterial activity, and at 26 °C overnight approx. 48-72 hr for anti-fungal activity. The Petri plates were examined for anti-bacterial activity after 18-24 h of incubation.

## Result and Discussion

The physical data of all synthesized compounds **(4a-g)** are shown in Table 1. In the elemental analysis, the percentage of C, H and N atoms are present in the range of ± 0.06 as shown in Table 1.

The IR spectrum of final compound **(4a)** exhibited the isatin carbonyl at 1743 $cm^{-1}$ and S-H of 5-mercapto oxadiazole at 2565 $cm^{-1}$, which confirms the formation of the final compound. In the $^1$HNMR (DMSO-$d_6$) spectrum of **4a**, all protons appeared at the expected chemical shift and integral values at δ 3.490 (s, H, -SH), 5.145 (s, 2H, N-$CH_2$), 6.726-7.438 (m, 8H, Ar-H).

The fragmentation pattern of representative compound **4a** presented in Figure 2, is an additional evidence for the proposed structure. The m/z ratio of 76 with 100% abundance is the base peak. The molecular

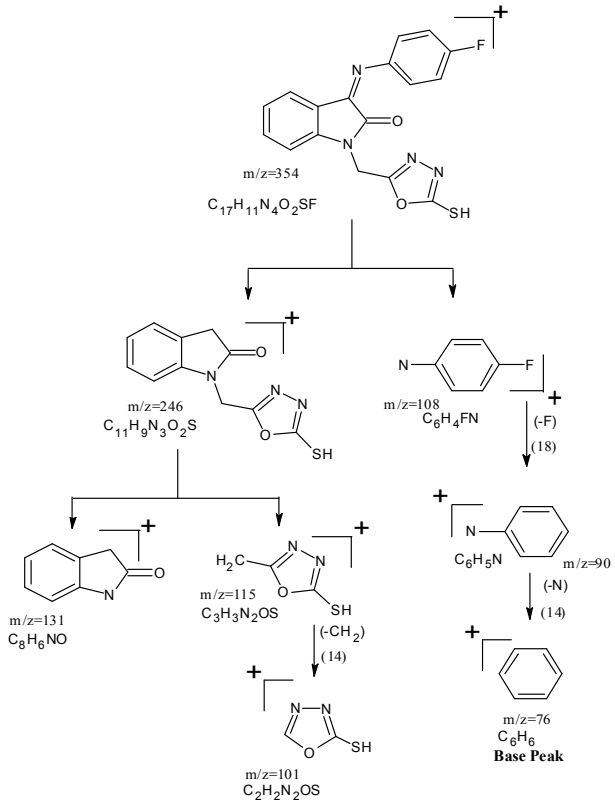



**Figure 2.** Mass fragmentation pattern of 4a.

**Table 2.** Biological activities of the compounds 4a-g at 200 µg/ml

| Sample code | R | R1 | Zone of inhibition (mm) | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Antimicrobial activity (200 µg/ml) | | | | Antifungal activity (200 µg/ml) | |
| | | | *S. aureus* | *B. subtilis* | *E. coli* | *P. vulgaris* | *C. albicans* | *A. niger* |
| 4a | F | H | 5 | 8 | NS | NS | NS | NS |
| 4b | F | Cl | 7 | 5 | NS | NS | NS | NS |
| 4c | F | F | 11 | 5 | 5 | 8 | 4.5 | NS |
| 4d | F | $CH_3$ | NS | 7 | 6 | 5 | 5 | NS |
| 4e | F | Br | 9 | NS | NS | 6 | 8 | NS |
| 4f | Cl | H | NS | 10 | 5 | 7 | 9 | NS |
| 4g | Br | H | NS | NS | NS | NS | 4 | NS |
| A | Norfloxacin | | 8 | 12 | 11 | 12 | ---- | ---- |
| B | Fluconazole | | ---- | ---- | ---- | ---- | 10 | 12 |

NS: Not significant

**Table 3.** Biological activities of the compounds 4a-g at 500 µg/ml

| Sample code | R | R1 | Zone of inhibition (mm) | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Antimicrobial activity (500 µg/ml) | | | | Antifungal activity (500 µg/ml) | |
| | | | *S. aureus* | *B. subtilis* | *E. coli* | *P. vulgaris* | *C. albicans* | *A. niger* |
| 4a | F | H | 25 | 27 | 15 | 15 | 23 | NS |
| 4b | F | Cl | 20 | 25 | 20 | 10 | 21 | NS |
| 4c | F | F | 35 | 22 | 25 | 24 | 28 | NS |
| 4d | F | $CH_3$ | 10 | 28 | 16 | 20 | 34 | 25 |
| 4e | F | Br | 27 | 19 | 15 | 20 | 30 | NS |
| 4f | Cl | H | 14 | 30 | 20 | 21 | 20 | 15 |
| 4g | Br | H | 12 | 16 | 10 | 15 | 25 | NS |
| A | Norfloxacin | | 20 | 20 | 32 | 32 | ---- | ---- |
| B | Fluconazole | | ---- | ---- | --- | ---- | 25 | 28 |

NS: Not significant

ion peak at 354 [$M^+$] is consistent with the molecular formula. The fragmentation peaks were observed at 246 ($C_{11}H_9N_3O_2S^+$), 131 ($C_8H_6NO^+$), 115 ($C_3H_3N_2OS^+$), 108 ($C_6H_4NF^+$), 101 ($C_2H_2N_2OS^+$), 90 ($C_6H_5N$), and 76 ($C_6H_4^+$) (100%).

All compounds were screened at the concentrations of 200 µg/ml and 500 µg/ml. The results of antimicrobial screening are presented in Tables 2 and 3. From the data presented, it is clear that **4a** is highly active against *Staphylococcus aureus* and *Bacillus subtilis*. In the case of *Escherichia coli* and *Proteus vulgaris*, compounds **4c** and **4f** are highly active. The compound **4d** exhibited significant activity against *Candida albicans* and *Aspergillus niger*. Compounds **4c** and **4e** were highly active against *Staphylococcus aureus and* **4b, 4d** and **4f** were highly active against *Bacillus subtilis*. In the case of gram-negative bacteria, **4d** showed moderate activity against *Proteus Vulgaris*. Similarly, compounds **4c, 4e, 4g** showed moderate activity in the case of *Candida albicans* Similar activity for *Aspergillus niger* was found for compound **4f**.

In summary, it can be concluded that remarkable inhibition is observed in compounds bearing $R_1$ = F, $CH_3$, and Br substituents.

## Acknowledgement

Authors are thankful to the Department of Microbiology, R.C.Patel, College of Arts, Science and Commerce, Shirpur, Dist Dhule, India for carrying out the anti-microbial activities.

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